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# INFLUENCE OF LIGHT AND PHOTOSYNTHESIS ON ALKALOID CONCENTRATION IN LARKSPUR

M. H. RALPHS,[1,*] G. D. MANNERS,[2] and D. R. GARDNER[1]

[1]*USDA/ARS Poisonous Plant Laboratory*
*1150 E. 1400 N., Logan Utah 84341*

[2]*USDA/ARS Western Regional Research Center*
*Albany California 94710*

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**Abstract**—Concentrations of toxic norditerpenoid alkaloids vary greatly in tall larkspur (*Delphinium barbeyi*) and may be influenced by environmental stress. We evaluated the effect of shade, darkness, and inhibition of photosynthesis on toxic alkaloid concentration. In plants treated with metribuzin to inhibit photosynthesis, alkaloid concentration increased, but dry weight of the plants decreased as growth ceased, leaving absolute alkaloid content similar to that of control plants. Short-term shade (70% reduction in sunlight for three days), dark treatments from leaves collected at night, and aluminum foil covered leaves all increased alkaloid concentration in comparison to untreated control plants. It appears that absolute amounts of alkaloids remained the same, but the mass of stressed plants declined as nonstructural carbohydrates were depleted, thus increasing the relative concentration of alkaloids. We conclude that norditerpenoid alkaloids in larkspur do not respond to short-term light stress. Alkaloid concentration was lower in larkspur plants growing beneath forest canopy and in potted plants in a long-term shade study (70% reduction in sun light for 21 days) than plants growing in open sunlight. Long-term shade may have reduced synthesis of norditerpenoid alkaloids, particularly in the earlier developmental stages of the plant. Shade stress or photosynthesis inhibition apparently did not increase norditerpenoid alkaloid synthesis, which contrasts with the carbon/nutrient balance theory of plant defense.

**Key Words**—*Delphinium barbeyi*, norditerpenoid alkaloids, methyllycaconitine, 14-deacetylnudicauline, environmental stress, shade, diurnal, dark, photosynthesis inhibition, metribuzin, carbon/nutrient balance theory.

*To whom correspondence should be addressed.



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## INTRODUCTION

Larkspur species (*Delphinium* spp.) contain norditerpenoid alkaloids that are acutely toxic to cattle (Olsen 1978). Larkspurs are also palatable (Pfister et al., 1988), thus causing severe poisoning problems on mountain rangeland (Nielsen and Ralphs 1988). Concentrations of toxic alkaloids are known to vary between species, locations, plants within a population, plant part, and phenological growth stage (Manners et al., 1993; Pfister et al., 1994; Ralphs et al., 1988, 1997). The reason why the toxin level varies is not known.

The resource availability theory of plant defense suggests that availability of nutrients in the environment is the major factor influencing the type and amount of defense compounds (Coley et al., 1985). This theory predicts that rapidly growing plants in resource-rich habitats contain low levels of highly mobile toxins, such as alkaloids and cyanogenic glycosides. These plants exhibit biochemical and morphological plasticity to allow them to take advantage of pulses in resource availability. Nitrogen (N) is taken up early in the growing season in excess of the plant's needs for growth. Excess N is available to be synthesized into N-based qualitative or toxic defense compounds that can be rapidly induced upon injury to protect the plant from further herbivory (Mooney et al., 1983).

The carbon/nutrient balance theory further explains changes in concentrations in defense compounds (Bryant et al., 1983, 1992). If light becomes limiting (i.e., shade or cloudy weather) to plants growing in nutrient-rich environments, the decline in photosynthesis may limit carbohydrates for growth and carbon-based defenses, but nutrient uptake continues, leaving excess N that could be shunted to N-based defense compounds such as alkaloids.

The objective of this study was to determine the influence of light and photosynthesis on norditerpenoid alkaloid concentration in tall larkspur (*Delphinium barbeyi*). The carbon/nutrient balance theory predicts that shade stress or photosynthesis inhibition should increase alkaloid concentration.

## METHODS AND MATERIALS

### *Larkspur Alkaloids*

The toxic compounds in larkspurs have been identified as norditerpenoid alkaloids (Figure 1). Alkaloids that contain the *N*-(methylsuccinimido) anthranilic ester group (referred to as MSAL alkaloids) are the most toxic (Manners et al., 1995), with methyllycaconitine (MLA) and 14-deacetylnudicauline (DAN) being the two most prominent toxic alkaloids in tall larkspur. The alkaloids MLA and DAN were extracted in ethanol and chloroform, then quantified by normal-phase, isocratic high-pressure liquid chromatography (HPLC) (Manners

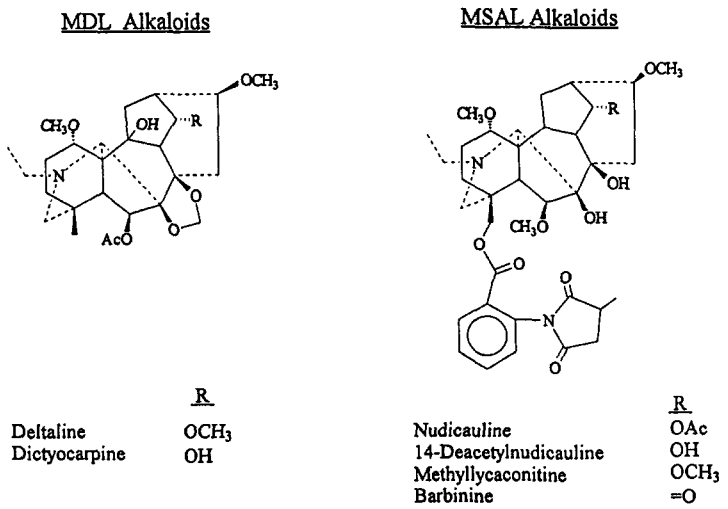


FIG. 1. Nornorditerpenoid alkaloids in larkspur species. The two major classes of alkaloids are methylenedioxylcoctonine (MDL) and *N*-(methylsuccinimido)anthranoyllycoctonine (MSAL) alkaloids.

and Pfisters, 1983) in the shade and dark stress studies. These alkaloids are of similar toxicity (Manners et al., 1995); therefore, they were summed to express toxic alkaloid concentration. In the study using metribuzin to inhibit photosynthesis, the MSAL fraction and total norditerpenoid alkaloid concentration [which includes MSAL and methylenedioxylycoctonine (MDL) type alkaloids] were measured by Fourier transform infrared spectroscopy (FTIR) (Gardner et al., 1997). They were extracted in chloroform and 1% $H_2SO_4$, and IR spectra were collected using a Nicolet Magna 550 FT-IR spectrometer (Nicolet Instrument Corp., Madison, Wisconsin). This new method used HPLC values from previous studies to calibrate the predictive equation. The alkaloids MLA and DAN comprise most of the MSAL fraction, therefore the MSAL fraction is also referred to as toxic alkaloids in the metribuzin study. Samples from the greenhouse studies were frozen and freeze-dried prior to extraction. Samples from the field were dried in a forced air oven at 60°C for 48 hr. A comparison was made between drying methods, and there were no differences in either toxic or total alkaloids.

### *Long-Term Shade Stress*

*Field Studies, 1992.* Samples of tall larkspur and duncecap larkspur (*D. occidentale*) were collected in conjunction with a large sampling program to

assess the toxicity of larkspur species and populations (Ralphs et al., 1997). Leaves from larkspur plans growing under conifer or aspen canopy, and adjacent plants growing in open sunlight were harvested at two-week intervals during the growing season. Tall larkspur samples were collected in a subalpine fir community 24 km east of Manti, Utah, at an elevation of 3000 m, and in an aspen community 46 km east of Salina, Utah, at an elevation of 3100 m. Duncecap larkspur samples were collected in aspen communities 32 km west of Oakley, Idaho, at 2500 m, and 28 km east of Jackpot, Nevada, at 2300 m. Samples were harvested around noon to reduce any effects of dirunal variation in alkaloid concentration. Five leaves from 30–50 plants growing in the sun, and five leaves from a similar number of plants growing in shade were harvested and composited into single samples (sun or shade treatments) for each time at each location. The samples were analyzed for toxic alkaloids by HPLC. The toxic alkaloid concentrations in shade and sun plants were compared by paired *t* tests.

*Potted Plants, 1993.* Tall larkspur plants were established from seed collected from the Manti, Utah, site and were grown in the greenhouse. After overwintering in a dark cold room (5°C), 30 plants were taken out on March 26, 1993, and cloned by splitting each plant in half. The roots were rinsed in a dilute Clorox solution, then dipped in a root-stimulating hormone solution. They were planted in a growing medium of equal parts of soil, periolite, and peat in 20-cm pots and allowed to grow in the greenhouse. These plants were fertilized biweekly with Peters 20-20-20 NPK garden fertilizer. In addition to producing clones, the newly potted plants prevented any suppression of alkaloid synthesis that may be inherent in root-bound potted plants (Baldwin, 1988). On May 10, 10 cloned pairs were placed outside to acclimate to outdoor growing conditions for three weeks. One of each cloned pair was then randomly selected and placed under a shade cloth canopy designed to filter out 70% of light. The other plant was left in the open sun. The plants were left in these treatments for three weeks. Midday photosynthetic photon flux (PPF) was measured by a Li-Cor LI-185B photometer and averaged 2000 $\mu$mol/m$^2$/sec in the open and 750 $\mu$mol/m$^2$/sec in the shade. At the end of three weeks, the leaves were harvested, frozen, freeze-dried, and ground to pass through a 1-mm screen in a Wiley mill. Alkaloids were extracted and individual toxic alkaloids were quantified by HPLC. Differences in toxic alkaloids between treatments were determined by paired *t* tests.

### *Short-Term Shade Stress*

*Potted Plants, 1994.* Cloned plants were overwintered in a dark cold room, then taken out and allowed to grow in the greenhouse during the spring. The foliage was clipped back to 2.5 cm from the soil surface, and 25 pairs of clones were placed outside to regrow and acclimate to the outside environment on May

13. They were allowed to grow for 25 days before being placed in the respective shade treatments for three days. As before, one of each clone was randomly selected and placed under 70% shade cloth, while the other was left in the open sun. Mean irradiation levels at mid day were 2067 $\mu$mol/m$^2$/sec in the open sun and 173 $\mu$mol/m$^2$/sec in the shade, and temperatures were 31°C and 25°C, respectively. Leaves were harvested from the plants at midday on the third day, frozen, freeze-dried, ground, and analyzed by HPLC.

*Potted Plants, 1995.* Cloned plants were overwintered in the cold room, then taken out in March and grown in the greenhouse. On June 21, 20 cloned pairs were clipped near the soil surface and then allowed to grow outside in open sunlight. One of each cloned pair was placed in the 70% shade treatment on July 26, then the leaves from both plants of the clone were harvested three days later, frozen, freeze-dried, and toxic alkaloids were measured by HPLC. Toxic alkaloids in shade and sun plants were compared by paired *t* tests.

### *Dark Stress on Field Plants, Salina 1994*

*Diurnal.* The study site was located near the 1992 Saline study site at 3000 m elevation. Larkspur plants were in the bud elongation stage of development in mid-July. Ten larkspur plants were selected; 10 stems from each plant were harvested at 05:00 hr (before dawn) for the dark treatment, and another 10 stems from the same plant were harvested at noon for the sun treatment. Leaves from the top two thirds of the stalks were plucked and placed in a separate paper bag for each plant. The samples were returned to the laboratory, dried in a forced-air oven, and then extracted and analyzed for toxic alkaloids by HPLC. The alkaloid concentration was analyzed by paired *t* tests comparing samples from the same plant during dark and sun periods.

*Aluminum Foil on Leaves.* The study site was near the diurnal study at Salina at 3200 m elevation. It was on a snowdrift site, and the larkspur plants were at a younger stage of development (late vegetative). Five plants were selected on each of four days (July 15, 16, 20, 21, 1994). Aluminum foil was placed on alternate leaves in the early afternoon, then all the leaves were harvested late the next afternoon. Covered leaves and leaves exposed to sunlight from each plant were placed in separate bags, dried in a forced-air oven, and analyzed by HPLC. Data were analyzed by paired *t* tests.

### *Metribuzin Treatment to Inhibit Photosynthesis*

*1994 Study.* The herbicide metribuzin [4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4H)-one] inhibits photo system II in the photosynthetic process (Devin et al., 1993). It not only stops photosynthesis, but toxic oxygen species are created, which in turn form caustic hydroxyl radicals that

rapidly disrupt cell membranes. Whereas the shade stress and dark treatments reduced or stopped photosynthesis and subsequent carbohydrate synthesis, metribuzin rapidly depletes energy pools and disrupts membranes of the chloroplast, thus stopping any biosynthetic pathways in the chloroplast. If alkaloid synthesis occurs in the chloroplast, we would expect this treatment to reduce alkaloid concentrations.

The site for the study was 50 km east of Salina, Utah, at 2700 m elevation. Four larkspur patches were split in half, and one half was randomly selected and sprayed with metribuzin at a rate of 140 g ai (active ingredient)/ha (2 oz/ac). A backpack sprayer was used with a 4-nozzle boom, putting out 173 liters of total spray solution per hectar. Five leaves were harvested from 30–50 plants in each plot on day 0 before spraying, and 1, 3, 7, and 14 days after treatment. The samples were oven dried and toxic and total alkaloids were estimated by Fourier transform infrared spectroscopy (FTIR). The concentrations of toxic and total alkaloids were compared by analysis of variance (ANOVA) in a split-plot design, with the treatments as the main plot and time the split plot.

*1995 Study.* The study site was in the next drainage valley 2 km west of the 1994 site in Salina Canyon. Forty larkspur plants were staked, and half were sprayed with metribuzin at the same herbicide concentration as used in the 1994 trial. Each plant was sprayed to wetness using a single solid cone nozzle. A single stalk from each plant was harvested on day 0 before treatment, then 1, 4, 7, and 14 days after treatment. The stalk was weighed when harvested and again after drying in a forced-air oven, and dry weight and percentage dry matter were calculated. The entire stalk, including stem, leaves, and head was ground and analyzed for alkaloids in order to determine the alkaloid concentration and absolute amount of alkaloid in the stalk. Toxic and total alkaloids were estimated by FTIR. Toxic and total alkaloid concentration was multiplied by the dry weight of each stalk to obtain absolute amount of alkaloids in each stalk. The alkaloid concentrations and absolute amounts of alkaloids were compared between the metribuzin-treated plants and untreated control plants in a repeated measures ANOVA over time.

### *Variability Among Plants*

The variability in toxic alkaloid concentration between individual plants was partitioned by analysis of variance (ANOVA) for the 1994 field diurnal and aluminum foil studies and in the 1995 metribuzin study to inhibit photosynthesis. The data for the diurnal and aluminum foil study were arranged in a simple randomized block design with plants serving as blocks. In the metribuzin study, the data were arranged in a randomized-block, split-plot design with individual plants as blocks and days as the split plot. The proportions of variation due to treatment, plant, and day (in the metribuzin study), were calculated by the ratio

of sum of squares for treatment, plant, and day to the total sum of squares (Coleman et al., 1987).

## RESULTS

### *Alkaloid Profile*

There was a marked difference in the amount and proportion of individual alkaloids between larkspur plants in pots started in the greenhouse and plants growing naturally in the field. Toxic alkaloids in potted plants in the 1993 and 1995 shade-stress studies were an order of magnitude lower than in field-grown plants in the 1992 and 1994 studies (Table 1). The concentraitons in potted plants were slightly higher in the 1994 shade study, but still substantially lower than in the field grown plants. The difference in toxic alkaloid concentration between field and potted plants is not likely due to the root-bound condition of the plants (Baldwin, 1988). The 1993 plants were newly split and had adequate room for growing roots. In a related study, newly cloned plants were actually lower in toxic alkaloids than plants growing in the same pot for three years (Ralphs, unpublished data). It is unlikely that larkspur alkaloids are produced in root tips and translocated up through the xylem to the foliar parts, as suggested by Baldwin (1991) in wild tobacco (*Nicotiana sylvestris*).

The composition of alkaloids also differed between potted plants and plants growing in the field. The concentration of DAN was similar in potted plants and those growing in the field (0.2–0.5 mg/g), but MLA increased from 0.2–0.9 mg/g in the potted plants to 5–10 mg/g in the field plants. The difference in toxic alkaloids between potted plants and those growing in the field was due almost entirely to the increase in MLA.

In the metribuzin studies, alkaloids were quantified by infrared spectroscopy (FTIR), which gives the toxic alkaloid fraction and total norditerpenoid alkaloid concentration. The toxic alkaloids comprised 40% of total norditerpenoid alkaloids at the two Salina sites.

### *Long-Term Shade Stress*

Leaves of tall and duncecap larkspur growing in open sunlight in the 1992 field study contained higher concentrations of toxic alkaloids than plants growing in the shade of either aspen or conifer ($P < 0.07$, Table 1). We speculate that reduction in light under tree canopy reduced synthesis of toxic alkaloids in the leaves. Other environmental differences between the shaded and open sites (temperature, soil type, fertility and moisture) may have also contributed to the difference in toxic alkaloids.

In the 1993 study, cloned larkspur plants grown in the open sun also had

TABLE 1. INFLUENCE OF SHADE AND DARK STRESS ON TOXIC ALKALOID CONCENTRATION IN LARKSPUR PLANTS

| Study | Species/location | Year | *N* | Conc. (mg/g) | | *P* |
|---|---|---|---|---|---|---|
| | | | | Sun | Shade or dark | |
| Long-term shade | | | | | | |
| Field | Tall | 1992 | 9 | 4.9 ± 1.3 | 4.0 ± 1.0 | 0.02 |
| | Duncecap | 1992 | 8 | 3.1 ± 0.7 | 2.3 ± 0.4 | 0.07 |
| 21-day shade | Potted plants | 1993 | 8 | 0.4 ± 0.07 | 0.24 ± 0.0 | 0.01 |
| Short-term shade | | | | | | |
| 3-day shade | Potted plants | 1994 | 24 | 1.1 ± 0.65 | 1.5 ± 0.91 | 0.05 |
| | Potted plants | 1995 | 19 | 0.24 ± 0.0 | 0.33 ± 0.0 | 0.06 |
| Dark stress | | | | | | |
| Diurnal | Salina | 1994 | 10 | 5.7 ± 0.8 | 6.4 ± 0.97 | 0.08 |
| Aluminum foil | Salina | 1994 | 20 | 5.3 ± 0.6 | 5.9 ± 0.7 | 0.02 |

a higher concentration of toxic alkaloids than those grown in shade for three weeks ($P = 0.01$, Table 1). Plants in the shade were developmentally younger, which should have made them higher in alkaloid concentration (Ralphs et al., 1997). This suggests that there may have been a real decline in toxic alkaloid concentration in long-term shade stressed plants. Majak et al. (1977) also reported that miserotoxin (3-nitropropanol) in timber milkvetch (*Astragalus miser* var. *serotinus*) was higher in plants growing in open grassland compared to plants growing under forest canopy.

*Short-Term Shade and Dark Stress*

Plants stressed for short periods of time in shade or darkness increased toxic alkaloid concentration. In the three-day shade studies in 1994 and 1995, shade stressed plants had higher toxic alkaloid concentrations than the plants growing in open sun ($P < 0.06$, Table 1). In the 1994 diurnal field study at Salina, plants harvested before dawn were higher in toxic alkaloid concentration than those harvested at noon although not by a statistically significant amount ($P = 0.08$, Table 1). Leaves in total darkness in the aluminum foil study also had higher mean toxic alkaloid concentration than those exposed to sunlight ($P = 0.02$, Table 1).

The slight increase in alkaloid concentration in these four studies could have been due to the relative decrease in biomass of the plants under stress. Unrestricted photosynthesis in plants exposed to full sunlight would continue to produce carbohydrates and other metabolites, thus increasing plant biomass and reducing the relative concentration of a constant amount of alkaloids. Restricted photosynthesis in shade and dark stressed plants may have reduced the relative biomass as carbohydrates were depleted or translocated out of the leaves, thus increasing the concentration of alkaloids. Smart et al. (1994) reported that total nonstructural carbohydrates (TNC) in wheat declined 50% from peak concentration during the day, to a low concentration at night. This accounted for a 15% reduction in dry weight of the shoot. Furthermore, the influence of shade within the wheat canopy (80% reduction in PPF from top to bottom) also reduced TNC 50% in bottom leaves compared to top leaves, resulting in a decrease of 5% in the dry weight of the leaves. The concentration of toxic alkaloids in our study increased an average of 11% in the dark treatments of the diurnal and aluminum foil field studies, and 39% in the shade studies using potted plants. If TNC depletion in larkspur leaves in dark and shade were similar to wheat, this reduction in mass could account for the relative increase in concentration of toxic alkaloids.

*Metribuzin Treatment*

Toxic and total alkaloid concentrations increased in plants treated with metribuzin over time, compared to a slight reduction in concentration in control

plants in both years ($P < 0.02$, Figure 2). However, there was no difference in absolute amount of toxic alkaloids in larkspur stalks between treatments ($P = 0.48$, Figure 3A). The absolute amount of alkaloid was calculated by multiplying the dry weight of the stalk (Figure 3C) by its toxic alkaloid concentration (Figure 3B). The toxic alkaloid concentration in control plants gradually declined over time (Figure 3B). This is the typical trend over the growing season (Man-

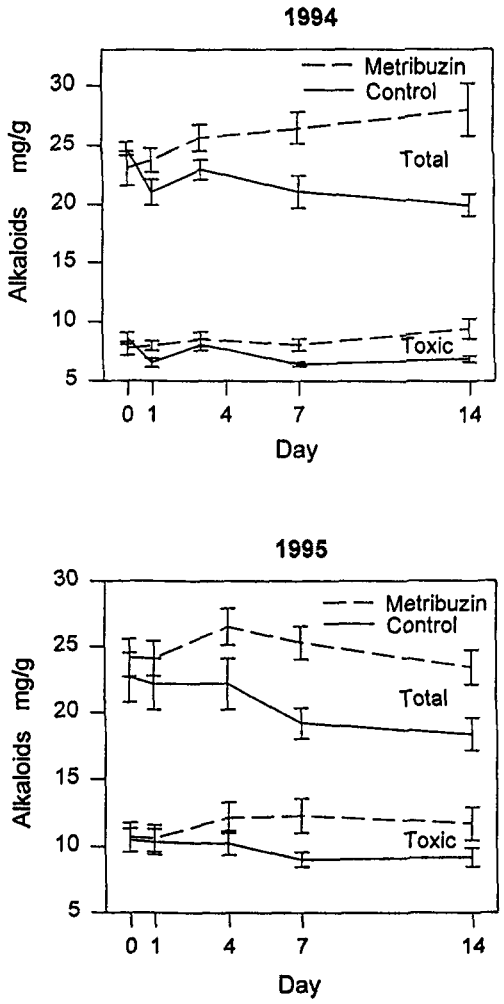


FIG. 2. Toxic and total alkaloid concentration in larkspur plants treated with metribuzin in the 1994 and 1995. Error bars are standard errors of the means.

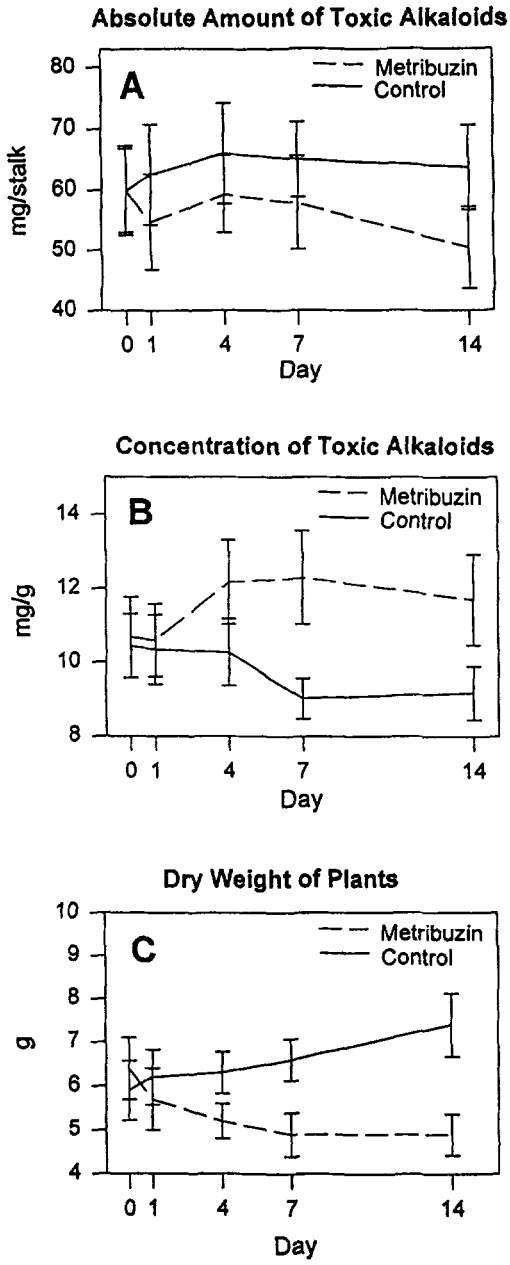


FIG. 3. (A) Absolute amount of toxic alkaloids, (B) concentration of toxic alkaloids, and (C) dry weight of larkspur stalks treated with metribuzin to inhibit photosynthesis, and untreated control plants. Error bars are standard errors of the means.

ners et al., 1993; Pfister et al., 1994; Ralphs et al., 1997). However, the dry weight of control plants increased as the plants continued to grow (Figure 3C), causing the absolute amount of toxic alkaloids to remain relatively constant (Figure 3A). In plants treated with metribuzin, toxic alkaloid concentration increased (Figure 3B), but dry weight of the stalk decreased (Figure 3C) as photosynthesis ceased and carbohydrate synthesis stopped. The plants did not desiccate; the percentage dry matter remained $<20\%$ and was similar to control plants. Thus, the absolute amount of alkaloid in metribuzin-treated plants remained similar to the controls, even though their alkaloid concentrations and dry weights changed in opposite directions. Total alkaloids responded in a similar manner (data not shown).

*Variation Among Plants*

We investigated the variation in toxic alkaloid concentration among individual plants by partitioning the ratio of sum of squares in the ANOVA. The difference in toxic alkaloid concentration among plants was much greater than the difference between treatments. In the 1994 diurnal and aluminum foil studies comparing samples from the same plant, differences between plants accounted for 95% and 96% of the variability in the statistical model, while treatment differences accounted for only 1%. Toxic alkaloid concentration among the plants ranged from 1 to 12 mg/g, compared to the average of 5 and 6 mg/g for dark and light treatments, respectively. In the 1995 metribuzin study, treatment accounted for 5% of variation, day (change over two weeks) accounted for 4%, but plant differences accounted for 51% of the variation. Toxic alkaloid concentrations ranged from 1 to 23 mg/g, compared with the treatment means of 9 and 11 mg/g for the control and photosynthesis-inhibited treatments, respectively. Manners and Pfister (1996) also reported large variation in alkaloid concentration between larkspur plants. Other studies reported great variation in toxin concentration among plants in a population (Vrieling et al., 1993; Van Dam and Vrieling, 1994; Zangerl and Berenhaum, 1990; Coleman et al., 1987).

## DISCUSSION

There was no difference in absolute amount of toxic and total alkaloids in larkspur stalks treated with metribuzin to inhibit photosynthesis and control plants. The increase in concentration of toxic and total alkaloids in plants treated with metribuzin was relative to the decline in the biomass of the plant, presumably because soluble carbohydrates were depleted and not replenished through photosynthesis. The lack of difference in absolute amount of alkaloids between control and metribuzin-treated plants and the lack of change in absolute amount of alkaloids over the 14-day experiment suggests that photosynthesis inhibition did not increase alkaloid synthesis.

Our results differ from those reported by Wink and Witte (1984) dealing with quinolizidine alkaloids in annual lupine (*Lupinus albus*). Synthesis of quinolizidine alkaloids occurred in chloroplast of leaves and was driven by light. Alkaloid concentration in leaves increased as light intensity increased and peaked at midday. Metribuzin disrupts chloroplast membranes. If norditerpenoid alkaloid synthesis occurred in chloroplasts, we would have expected the alkaloid concentration to decline in plants treated with metribuzin. Since the alkaloid concentration increased (but the absolute amount of alkaloids remained constant), we conclude that norditerpenoid alkaloids are not synthesized in chloroplasts during the bud elongation growth stage, the time at which this experiment was conducted.

In larkspur plants stressed by three-day shade or dark treatments, the slight increase in toxic alkaloid concentration probably resulted from the relative decrease in nonstructural carbohydrates as they were utilized or translocated out of the leaves.

Long-term shade treatments on potted larkspur plants and larkspur plants growing under shade in the field had lower toxic alkaloid concentrations than larkspur plants growing in open sunlight. These plants were developmentally younger than those in the sun and should have had higher concentrations of alkaloids. It is feasible that reduced light and cooler temperatures in shaded environments may reduce synthesis of norditerpenoid alkaloids in larkspur early growth, resulting in lower levels of alkaloids as the plants mature.

The combined results of our experiments do not fit the carbon/nutrient balance theory of plant defense compounds (Bryant et al., 1983, 1992), which predicts that as photosynthesis declines, resources are shunted to synthesis of N-based defense compounds. Short- and long-term shade, dark stress, or photosynthesis inhibition apparently did not increase alkaloid synthesis. Furthermore, simulated storms (Ralphs, unpublished data) and insect damage from the larkspur mirid [*Hopplomachus affiguratus* (Heteroptera: Miridae)] (Ralphs et al., 1998) did not increase alkaloid levels. It appears that external environmental stresses do not affect norditerpenoid alkaloid synthesis in larkspur.

However, the concentrations of toxic alkaloids in tall larkspur decline dramatically over the growing season, form a high of 1.2% of dry weight in new early growth, to a low of 0.2% in mature plants (Ralphs et al., 1997). Gershenzon (1994) stated that the enzymes required for synthesis of some secondary compounds are only active for short periods in the plant's development. He reported monoterpene biosynthesis in peppermint (*Mentha piperita*) was restricted to a brief period during the first two weeks of leaf ontogeny. Enzymes for the synthesis of indole alkaloids in periwinkle (*Catharanthus roseus*) (DeLuca et al., 1988), and cyanogenic glycosides in sorghum (*Sorghum bicolor*) (Halkier and Moller 1989) were only present in early growth. Other studies reported early synthesis of secondary compounds, such as purine alkaloids from *Coffea arabica* (Frischknecht et al., 1986), alkaloid synthesis in *Cinchona* (Aerts et al., 1991),

caffeine in tea leaves (Fujimori et al., 1991), and glucoinsulates in rape leaves (Porter et al., 1991). Lerdau et al. (1994) cited several examples where monoterpene concentrations were highest in small expanding leaves. The results from these examples from the literature, as well as the results from our experiments, do not fit the growth/differentiation balance theory of plant defense (Lorio, 1986), in which secondary compound synthesis occurs during later periods of cell differentiation, not in the early growth stages of cell division and elongation.

We propose the hypothesis that norditerpenoid alkaloids in larkspur are synthesized during early growth and that synthesis slows down and stops as the plants continue to grow. The constant amount of alkaloids is then diluted as the biomass of the plant increases as it continues to grow, thus accounting for the observed decline in both toxic and total alkaloid concentration as the growing season progresses. The existing alkaloids in the plant may be translocated to the seeds as they mature, thus accounting for the observed increase in toxic alkaloids in pods (Pfister et al., 1994). Environmental conditions during this early growth period may influence the subsequent alkaloid level, but environmental changes throughout the rest of the season would not affect alkaloids. There still remains a substantial amount of variation in alkaloid content among plants. This may be due to genetics or to differences among microsites during the early growth period.

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